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Resilience of soil functions to transient and persistent stresses is improved more by residue incorporation than the activity of earthworms

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Abstract

The development of soil sustainability is linked to the improved management of soil biota, such as earthworms, and crop residues to improve soil physical structure, enhance microbial activities, and increase nutrient cycling. This study examined the impacts of maize residue (65.8 C/N ratio, dry biomass 0.75 kg m⁻²) incorporation and earthworms (70 g *Metaphire guillelmi* m⁻²) on the resistance and resilience of soil C and N cycling to experimentally applied stresses. Field treatments were maize residue incorporation, maize residue incorporation with earthworm addition, and an unamended control. Resistance and resilience of C mineralization, ammonia oxidation, and potential denitrification were investigated over 28 days following a persistent stress of Cu (1 mg Cu soil g⁻¹) or a transient heat stress (50 °C for 16 hours). The results indicated that C mineralization was more resistant and resilient than ammonia oxidation and denitrification to either a persistent Cu or a transient heat stress. The

26 application of maize residues significantly increased soil microbial biomass, C mineralization,
27 ammonia oxidation and potential denitrification compared with the unamended control. Maize
28 residues significantly improved the resistance and resilience of N processes to Cu and heat
29 stress. The presence of earthworms significantly increased potential denitrification but had
30 limited positive effect on functional resistance and resilience. This study suggested crop
31 residue incorporation would strongly increase soil functional resistance and resilience to
32 persistent and transient stresses, and thus could be a useful agricultural practice to improve
33 soil ecosystem sustainability.

34 **Keywords:** crop residue, soil fauna, C mineralization, ammonia oxidation, denitrification

35 **1. Introduction**

36 Increasing soil degradation has raised awareness of soil sustainability of which a central
37 component is the capability to withstand (resistance) and recover (resilience) from
38 environmental stresses (Griffiths and Philippot, 2013). So much so that a global resilience
39 programme in response to land use pressures has been suggested (Smith et al., 2016). Soil
40 microorganisms play a central role in conferring resistance and resilience, through their
41 central role within the soil food web and sensitivity to agricultural practices (de Vries and
42 Shade, 2013). Crop residue amendment would increase soil organic matter (SOM), the
43 decomposition of which provides nutrients and energy to support the growth and succession
44 of soil biota (Shade et al., 2012). Increased SOM also leads to improved soil physical
45 properties (Diacono and Montemurro, 2010), and accelerated carbon (C) and nitrogen (N)
46 cycling (Turmel et al., 2015). Thus, SOM may be an important resource to strengthen the
47 resistance and resilience of soil ecosystem (Lal, 2015).

48 Improving the management of soil biota, such as earthworms, in agroecosystem is an integral
49 part of sustainable management (Fonte and Six, 2010) especially as biotic interactions of the
50 soil food web are a critical determinant of soil function, including resistance and resilience (de

51 Vries and Wallenstein, 2017). Of the three ecological groups of earthworms: anecics build a
52 relatively permanent vertical burrow system and feed on organic matter collected from the
53 soil surface, epigeics live and feed within the soil matrix creating horizontal burrows, and
54 endogeics inhabit the surface layers of soil consuming fresh organic matter (Brussaard et al.,
55 2012). To varying extents earthworms mix organic matter into the soil, influence soil
56 aggregation and porosity (Fonte and Six, 2010), gas diffusion and soil water retention, and
57 soil microbial community structure (Bernard et al., 2012). The availability and composition of
58 substrate provided by crop residues affects earthworm diet, behaviour and growth (Brussaard
59 et al., 2012; Zheng et al., 2018). Experiments have shown that the interaction between
60 earthworms and plant residues affects soil functions. Thus, earthworms regulated the ratio of
61 C- to N- degrading enzyme activities during crop residue decomposition in a laboratory
62 experiment (Zheng et al., 2018). Aspects of the interaction between added crop residues and
63 earthworms have also been explored in a long-term field trial of a wheat-rice cropping system
64 in sub-tropical China (Tao et al., 2009). Results showed that the presence of earthworms
65 further enhanced protease and alkaline phosphatase activities in soil with incorporated maize
66 residue (Tao et al., 2009). A comparison of bacterial community structure in the same field
67 trial (Gong et al., 2018), showed that residue incorporation had significant effects on bacterial
68 community structure and that earthworms increased the ratio of Proteobacteria to
69 Acidobacteria (indicative of high nutrient turnover). Earthworms also increased the
70 connection between taxa, which is taken as an indicator of compositional resilience (Dunne et
71 al., 2002). The interaction between plant cover and earthworms on soil resistance and
72 resilience was explored in a short-term greenhouse experiment, which revealed that plants
73 rather than earthworms increased resistance and resilience to soil compaction (Griffiths et al.,
74 2008).

To further explore such interactions, we used samples from a long-term field experiment to determine whether amendments with maize residues and earthworms affected the functional resistance and resilience of soil. We quantified changes in C mineralization, ammonia oxidation and potential denitrification rates immediately after heat- (short-term transient) and Cu- (long-term persistent) induced stress and during subsequent recovery over 28 days after Griffiths et al. (2001). Because of the identified effects of earthworms and maize residues to alter microbial community composition and increase C and N cycling in the field experiment (Gong et al., 2018), we hypothesised that soil amended with maize residues and earthworms would have greater resistance and resilience than soil amended with maize residues alone.

2. Materials and methods

2.1 Study site and soil samples

A field trial at the experimental station of Nanjing Agricultural University (China, 118°47'E, and 32°03'N) was established in 2001. In each plot, there were three treatments as described by Tao et al. (2009): maize residues (*Zea mays* L.) incorporated into soil, maize residues incorporated into soil with earthworm (*Metaphire guillelmi*) addition, and a control with no additions. Each treatment had three replicate plots, arranged in a completely randomized experimental design. Earthworms were monitored after every harvesting stage annually and were added if necessary to maintain a density of 70 g earthworm m⁻². This earthworm is the dominant species in this area, commonly found in disturbed arable soil and its behaviour shows it to be endogeic (Gong et al., 2018). Maize residues (0.75 kg m⁻² air-dry weight, chopped < 2 cm) containing 7.96 g N kg⁻¹, 2.85 g P kg⁻¹, 10.67 g K kg⁻¹, and 65.8 C/N ratio were applied to the appropriate plots at the beginning at rice and wheat growth period every year.

The soil, classified as an Orthic Acrisol, was sampled in May 2016. From each plot, three surface soil samples (0-20 cm depth) approximately 10 kg in weight were randomly sampled

100 and mixed thoroughly. The soil had a pH (H₂O) of 8.25 and contained 5.86 g C kg⁻¹ and 0.71
101 g N kg⁻¹ soil (Shu, 2018). Soil microbial biomass carbon (MBC) was analysed by chloroform
102 fumigation (Vance et al., 1987). Mineral N (NO₃⁻ and NH₄⁺) was extracted by shaking with 2
103 M KCl for 1 hour and analysed using a continuous flow analyser (Skalar San++ 4800,
104 Netherlands). Dissolved organic carbon (DOC) was extracted following the method of Ghani
105 et al. (2003) and analysed using a TOC analyser (Dohrmann DC-80, UK).

106 **2.2 Resistance and resilience assay**

107 Soils from all the treatments were packed to a bulk density of 1.1 g cm⁻³ and incubated for 7
108 days with a water content of 60% water-filled pore space (WFPS) at 20 °C prior to analysis.
109 The stresses imposed followed Griffiths et al. (2001) and were: Cu (1 mg Cu soil g⁻¹) to
110 provide a persistent stress; and heat (50 °C for 16 hours) to provide a transient stress. For the
111 heat stressed soil, a preliminary test (Supplementary material 1) indicated that the temperature
112 of 40 °C that has been typically applied in studies on temperate soils (Griffiths et al., 2001)
113 was not a sufficient stress for these subtropical soils because of their great adaptation to a
114 relatively high temperature (Table S1), as also found by Zhang et al. (2010). For each soil,
115 aliquots were exposed to either a stress (heat or Cu) or were unstressed as a control, with six
116 replicates for each field treatment and laboratory applied stress. Each aliquot contained 220 g
117 dry-weight equivalent of soil (bulk density 1.1 g cm⁻³) in a 500 ml capacity polypropylene pot.
118 Six replicate aliquots of each stressed- soil were prepared by adding 2.2 ml of 1.57 M
119 CuSO₄·5H₂O to obtain a concentration of 1 mg Cu soil g⁻¹; or 2.2 ml of sterile distilled water
120 to both the heat-stressed and unstressed (control) soils. All the aliquots were then sealed with
121 parafilm to exchange air but prevent any water loss. The heat- stressed soils were then
122 incubated at 50 °C for 16 hours, while both Cu-stressed and unstressed soil were incubated at
123 20 °C for 16 hours. All aliquots were then incubated at 20 °C for the remainder of the
124 resilience assay.

125 To facilitate temporal description, day 0 was defined as the time when Cu or heat was applied.
 126 Subsamples were taken for the analysis of microbial functions at intervals of 1, 3, 7, 14 and
 127 28 days after the stresses were imposed. C mineralization was measured by the emission of
 128 CO₂ after 24 hours following the addition of 120 µl of organic C compounds which provides
 129 50 mg C ml⁻¹ and 9.72 mg N ml⁻¹ to a 2 g of soil (Shu, 2018). Ammonia oxidation was
 130 determined by the chlorate inhibition method (Groffman 1985). Potential denitrification was
 131 determined following anaerobic incubation of 20 g soil in the presence of 10% (v/v) acetylene
 132 (Shu, 2018).

133 **2.3 Data analysis**

134 A linear mixed effect model was fit in the “lme4” package for the “R” statistical programme
 135 (version 3.5.2) using the “lmer” function (Bates et al., 2019). Effects of fixed term and
 136 random term on C mineralization, ammonia oxidation and potential denitrification were
 137 analysed. Fixed terms were field treatment, stress, time, and their interaction (treatment ×
 138 stress × time). The replicate plot was considered a random term.
 139 Stability $f(t)$ was calculated as the change in biological functions of the stressed soil (β)
 140 compared with the unstressed control (α) at day t (Equation 1) (Zhang et al., 2010):

$$f(t) = \frac{\beta_t}{\alpha_t} \times 100 \quad (1)$$

141 Resistance was defined as the stability measured at day 1 after perturbation (Equation 2),
 142 while resilience was estimated as the integrative stability after day 1 up to 28 days following
 143 stress (Equation 3) (Shu, 2018).

$$Resistance = \frac{\beta_1}{\alpha_1} \times 100 \quad (2)$$

$$Resilience = \int_1^{28} f(t) dt / (28 - 1) \quad (3)$$

144 **3. Results and discussion**

145 Maize residue incorporation significantly increased the concentrations of dissolved organic C
 146 and microbial biomass C (Table 1). The linear mixed effect model demonstrated that maize
 147 residue incorporation significantly ($P < 0.001$) increased C mineralization, ammonia
 148 oxidation and denitrification (Table S3). These results are consistent with previous studies
 149 that maize residue addition increased microbial biomass C (Tao et al., 2009) and promoted C
 150 sequestration (Shu et al., 2015). An increased supply of nutrients, such as from the maize
 151 residues, can hasten microbial growth (Henderson et al., 2010), sustain microorganisms and
 152 enhance microbial activities (Shade et al., 2012).

153 When soil was stressed by either Cu or heat, C mineralization in all the treatments was more
 154 resistant and resilient than ammonia oxidation and potential denitrification (Table 2). This is
 155 consistent with several studies which demonstrated that N processes are more susceptible to
 156 external stresses than C processes (Bissett et al., 2013; Morillas et al., 2015). This may be
 157 because the microbial community carrying out C mineralization is more diverse and more
 158 functionally redundant than specialized microbial populations performing ammonia oxidation
 159 and denitrification (Philippot et al., 2013). Denitrification was particularly susceptible to the
 160 applied stresses, with resistance and resilience often less than 20% (Table 2), as also
 161 previously was shown for both Cu (Magalhães et al., 2007) and heat (Wertz et al., 2007).

162 We found that maize residue incorporation significantly ($P < 0.05$) increased the resistance
 163 and resilience of ammonia oxidation and potential denitrification to Cu (Table 2). This could
 164 be attributed to the enhanced microbial biomass (Table 1), as well as the buffering effects by
 165 adsorption or chelation of Cu^{2+} by organic matter which diminishes the bioavailability and
 166 toxicity of Cu to microorganisms (Degryse et al., 2009). The bioavailability of Cu in soil with
 167 incorporated crop residue was likely to be significantly less than in the unamended soil one
 168 day after Cu addition (Navel et al., 2010). Crop residues serve as an energy and nutrient
 169 source for microorganisms to accelerate microbial community succession and so increase

170 microbial biomass (Brandt et al., 2010). Access to a favourable resource is important for the
171 degree of recovery and the time that microorganisms take to recover (Placella et al., 2012).
172 That organic amendments improved soil functional resistance and resilience to Cu has been
173 reported previously for C mineralization in temperate soils (Gregory et al., 2009). In contrast,
174 we observed that crop residue incorporation in this soil decreased resistance and resilience of
175 C mineralization to Cu (Table 2). We saw that C mineralization in soil with incorporated crop
176 residue decreased significantly after 3 days incubation in both the unstressed and the Cu
177 stressed soil (Table S2). This could be related to the depletion of available nutrients. The
178 different impacts of residue on the resistance and resilience of C and N processes could also
179 be ascribed to the different stress-sensitivity and distinct microbial characteristics between C
180 and N processes.

181 All the measured microbial functions were resilient to heat, especially ammonia oxidation and
182 potential denitrification in the soil with incorporated maize residue (Table 2). Heat leads to
183 the death of heat-sensitive microorganisms, such as proteobacteria which had a low resistance
184 to heat stress (Frenk et al., 2017). In recovering from a transient heat stress, the attributes of
185 the microbial communities, mixtrophs and intrinsic growth rate, determine microbial use of
186 available C to reproduce and recolonize niches rapidly (Shade et al., 2012). Necromass, such
187 as dead microbial cells induced by the heat stress, also provides a rapidly mineralised
188 substrate that is easily accessible to free-living microorganisms (Drigo et al., 2012). A
189 previous study has demonstrated that bacterial communities could recover to its original
190 structure from a transient heat stress, but not from a persistent Cu stress (Shu, 2018). Routine
191 successional trajectories of microbial communities may be altered differently by different
192 stresses (de Vries and Shade, 2013), and gradual shifts of microbial community may be the
193 result of long-term adaptations to the persistent Cu stress.

194 In the soils with incorporated maize residues, earthworms significantly increased potential
195 denitrification (Table S3). Earthworms gut, casts and drilospheres are hotspots of
196 denitrification, and thus contribute to high emission of N₂O (Lubbers et al., 2013). However,
197 earthworm presence had few significant additional impacts on C mineralization and ammonia
198 oxidation (Table S3). The different response between soil functions to earthworms unveils
199 that their underlying microorganisms may be influenced by earthworms differently. For
200 example, in a northern temperate forest in USA, earthworms enhanced cellulolytic enzyme
201 activity and shifted soil microbial composition away from fungi and towards bacteria
202 (Dempsey et al., 2013). Previous studies, at the site where soils were collected for this
203 experiment, demonstrated that earthworms significantly changed the composition and
204 connectance of the microbial community (Gong et al., 2018) and soil enzyme activities (Tao
205 et al., 2009) when maize residues were incorporated. The lack of significant earthworm effect
206 on resistance and resilience in this experiment, suggests that these changes were not enough to
207 affect the stability of the soil. The effects of earthworms may also be overwhelmed by
208 residues, further study should include a treatment of earthworms alone without residue
209 amendment. The small effect of earthworms could result if not all the measured soils had
210 transited through the earthworm gut, because microorganisms and their activities can be
211 stimulated by earthworm mucus (Bernard et al., 2012). This study only focused on the bulk
212 soil, however, Gong and colleagues (unpublished data) have found a significant effect of
213 earthworms on the microbial community associated with soil aggregates. Therefore, it would
214 be interesting to explore how soil resistance and resilience changes at an aggregate scale.

215 In conclusion, C and N processes responded differently to imposed stresses, so it is important
216 that assays of resilience explore multiple functions and potential disturbances. Soil functions
217 are less likely to recover from a persistent stress (e.g. Cu) than a transient stress (e.g. heat),
218 but transient stresses can still result in a prolonged degradation to soil functions. Stresses

219 associated with climate change, such as the frequency of long hot periods, drought or flooding
220 could affect soil for a period after the stresses are removed. The important role of earthworms
221 in ecosystems is widely recognised, however, in this example of a disturbed agricultural soil
222 crop residue addition as a management option was more important than having earthworms
223 present for restoring soil resistance and resilience. Although further research is required
224 across a wider range of soils and with more types of residues, our findings suggest that
225 applying crop residues to a degraded agricultural soil is a primary driver in the recovery of
226 functions like C and N cycling that underpin productivity and sustainability.

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Table 1 Soil properties sampled at day 0 before stress imposed and the average functions in the unstressed soil over 28 days. MBC- microbial biomass carbon ($\mu\text{g C dry soil g}^{-1}$); NO_3^- - the total concentration of nitrate ($\mu\text{g N dry soil g}^{-1}$); NH_4^+ - the amount of ammonia N ($\mu\text{g N dry soil g}^{-1}$); DOC- dissolved organic carbon ($\mu\text{g C dry soil g}^{-1}$). C mineralization ($\mu\text{g C soil g}^{-1} \text{ h}^{-1}$), ammonia oxidation ($\mu\text{g N soil g}^{-1} \text{ d}^{-1}$), potential denitrification ($\text{ng N soil g}^{-1} \text{ h}^{-1}$) are the average in the unstressed soils during 28 days. Means \pm standard deviations followed by the same lowercase letters in the same column are not significantly ($P < 0.05$) different.

Treatment	MBC	NO_3^-	NH_4^+	DOC	C	Ammonia	Potential
					mineralization	oxidation	denitrification
Control	44.8 \pm 19 c	18.11 \pm 4 b	0.58 \pm 0.1 a	187.6 \pm 6 b	17.3 \pm 0.6 b	81.5 \pm 1.9 b	255 \pm 15 b
Residue	275.3 \pm 28 a	32.43 \pm 4 a	0.67 \pm 0.01 a	338.5 \pm 10 a	31.1 \pm 1.1 a	130.5 \pm 2.5 a	1437 \pm 134 a
Residue + Earthworms	199.9 \pm 31 b	31.75 \pm 2 a	0.67 \pm 0.03 a	310.2 \pm 7 a	29.6 \pm 1.3 a	129.3 \pm 2.4 a	1432 \pm 69 a

Table 2 The resistance and resilience of C mineralization, ammonia oxidation and potential denitrification to Cu and heat. Means±standard deviations followed by the same lowercase letters in the same indicator are not significantly ($P < 0.05$) different. Field treatments were the control without any additions, maize residue incorporation, and maize residue incorporation and earthworm addition.

Treatment	C mineralization		Ammonia oxidation		Potential denitrification	
	Resistance (Cu)	Resilience (Cu)	Resistance (Cu)	Resilience (Cu)	Resistance (Cu)	Resilience (Cu)
Control	87±4 a	67±1 a	30±3 b	30±2 c	15±1 a	5±1 b
Residue	74±3 b	59±2 b	46±2 a	48±1 a	17±2 a	8±1 a
Residue + Earthworms	69±3 b	62±2 b	45±1 a	40±2 b	7±1 b	9±1 a
Treatment	C mineralization		Ammonia oxidation		Potential denitrification	
	Resistance (Heat)	Resilience (Heat)	Resistance (Heat)	Resilience (Heat)	Resistance (Heat)	Resilience (Heat)
Control	61±3 a	77±2 a	30±2 b	60±2 b	18±4 a	34±4 b
Residue	55±1 a	68±3 b	44±4 a	77±2 a	6±1 b	49±3 a
Residue + Earthworms	59±2 a	75±2 a	36±1 b	76±3 a	3±1 b	45±4 ab

SUPPLEMENTARY MATERIALS

Resilience of soil functions to transient and persistent stresses is improved more by residue incorporation than the activity of earthworms

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Supplementary material 1: Preliminary Experiment

Soils from the treatments of control and maize residue incorporated and earthworm added were packed to a bulk density of 1.1 g cm⁻³, and incubated for 7 days with a water content of 60% water-filled pore space (WFPS) at 20 °C prior to the preliminary test. For each soil, aliquots were exposed to either a stress (heat at 40 °C or 50 °C) or were unstressed as a control, with three replicates for each treatment and stress. Each aliquot contained 220 g dry-weight equivalent of soil in a 500 ml capacity pot. Three replicate aliquots of each stressed- soil were prepared by adding 2.2 ml of sterile distilled water to both the heat-stressed and unstressed control soils. All aliquots were then sealed with parafilm to exchange air but prevent any water loss. The heat- stressed soils were then incubated at either 40 or 50 °C for 16 hours, while the unstressed soils were incubated in a moist atmosphere at 20 °C for 16 hours. All aliquots were then incubated at 20 °C for the remainder of the same resilience assay. C mineralization, ammonia oxidation and potential denitrification were measured over 7 days following stress. The methods of C mineralization, ammonia oxidation and potential denitrification are described in main text.

Table S1 C mineralization, ammonia oxidation and potential denitrification at different field treatments and stresses. Means \pm standard error of means. Field treatments were the control without any additions, and maize residue incorporation and earthworm addition.

Treatment	C mineralization ($\mu\text{g C soil g}^{-1} \text{ h}^{-1}$)				
	Day 1	Heat 50°C	Unstressed	Day 7	Unstressed
Control	Heat 40°C	11.9 \pm 1.3	20.7 \pm 1.5	Heat 40°C	Heat 50°C
Residue + Earthworms	16.4 \pm 0.6	22.2 \pm 0.1	34.9 \pm 2.6	12.4 \pm 0.1	9.5 \pm 0.1
	28.7 \pm 1.4			17.7 \pm 0.4	14.2 \pm 0.1
					18.8 \pm 0.2
Treatment	Ammonia oxidation ($\mu\text{g N soil g}^{-1} \text{ d}^{-1}$)				
	Day 1	Heat 50°C	Unstressed	Day 7	Unstressed
Control	Heat 40°C	33.4 \pm 0.6	95.4 \pm 2.3	Heat 40°C	Heat 50°C
Residue + Earthworms	88.6 \pm 4.6	62.1 \pm 5.2	126.0 \pm 1.3	93.1 \pm 1.0	51.9 \pm 2.0
	128.1 \pm 3.0			130.3 \pm 0.3	95.4 \pm 1.3
					136.3 \pm 1.1
Treatment	Potential denitrification ($\text{ng N soil g}^{-1} \text{ h}^{-1}$)				
	Day 1	Heat 50°C	Unstressed	Day 7	Unstressed
Control	Heat 40°C	44 \pm 14	268 \pm 83	Heat 40°C	Heat 50°C
Residue + Earthworms	247 \pm 10	28 \pm 8	1381 \pm 45	271 \pm 42	100 \pm 85
	959 \pm 31			1200 \pm 44	516 \pm 171
					328 \pm 16
					1224 \pm 71

Table S2 C mineralization, ammonia oxidation and potential denitrification at 1, 3, 7, 14 and 28 days after Cu, heat or no stress. Field treatments were the control without any additions, maize residue incorporation, and maize residue incorporation and earthworm addition. Means \pm standard error of means.

C mineralization (μg C soil g ⁻¹ h ⁻¹)				Ammonia oxidation (μg N soil g ⁻¹ d ⁻¹)				Potential denitrification (ng N soil g ⁻¹ h ⁻¹)			
Unstressed											
Day	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue + Earthworm
1	17.2±0.6	37.4±0.9	36.7±0.8	84.8±1.9	132.7±2.1	132.3±2.2	210.0±4.6	867.0±59.5	1652.0±46.4		
3	18.2±0.4	35.9±1.1	36.4±1.5	89.0±2.9	133.1±4.1	135.9±1.9	71.0±12.0	1082.0±143.9	959.0±63.1		
7	17.6±0.9	29.7±1.7	26.1±2.5	83.4±2.3	130.2±2.0	129.2±2.6	350.0±12.1	1644.0±180.2	1342.0±110.3		
14	16.6±0.5	27.1±1.2	25.0±0.8	82.4±1.3	129.4±1.1	127.9±2.7	201.0±21.1	1410.0±152.1	1288.0±51.5		
28	16.9±0.6	25.1±0.8	24.0±1.1	68.1±1.1	126.8±3.4	121.0±2.5	441.0±26.9	2179.0±137.8	1920.0±74.9		
Cu-stressed											
Day	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue + Earthworm
1	14.9±0.6	27.8±1.1	25.5±1.3	25.4±2.4	61.7±3.7	59.0±1.4	32.3±1.7	141.5±11.3	118.7±14.7		
3	13.1±0.4	23.9±0.6	24.7±1.8	27.9±2.0	75.3±3.5	63.6±3.9	12.4±1.2	53.5±7.5	30.7±4.5		
7	11.0±0.4	16.3±0.9	16.9±1.1	26.7±4.5	63.3±2.2	58.5±5.2	11.1±3.3	103.2±19.4	115.2±10.8		
14	10.6±0.4	15.1±0.5	14.2±0.8	24.1±3.2	63.0±2.2	47.6±4.2	6.1±1.2	149.1±18.9	122.6±16.7		
28	12.0±0.2	15.0±0.4	16.2±0.9	20.9±2.3	55.8±3.7	44.5±0.9	10.7±1.3	142.7±44.5	181.5±44.1		
Heat-stressed											
Day	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue + Earthworm
1	10.3±0.3	20.6±0.5	21.7±0.5	25.0±1.4	58.8±5.0	47.2±1.3	36.8±8.0	53.7±4.6	48.4±5.4		
3	11.7±0.2	18.7±0.6	18.0±0.7	49.7±2.5	106.7±4.4	106.3±4.2	20.6±2.9	114.5±35.5	89.8±17.2		
7	14.1±1.2	18.4±1.0	19.9±0.3	47.0±2.0	95.8±4.4	93.3±1.8	81.5±19.6	788.6±94.2	678.3±88.3		
14	13.1±0.2	18.0±0.8	19.3±0.4	50.1±2.2	102.9±2.8	99.0±4.5	70.3±13.1	864.2±123.8	689.4±85.8		
28	13.9±0.4	20.8±0.9	20.1±0.7	45.4±1.3	100.6±4.7	97.7±3.1	196.6±19.8	1303.9±150.6	966.4±119.4		

Table S3 Coefficients (estimate \pm standard deviation) and R^2 values for the linear mixed effect model to evaluate the main and interaction effect of field treatment, stress and time on C mineralization, ammonia oxidation, and potential denitrification. Contributions of all the fixed effects and random effects to the complete model were evaluated as R^2 . *, **, *** indicates the significance at $P < 0.05, 0.01, 0.001$, respectively.

	Fixed term	C mineralization ($\mu\text{g C soil g}^{-1} \text{ h}^{-1}$)	Ammonia oxidation ($\mu\text{g N soil g}^{-1} \text{ d}^{-1}$)	Potential denitrification ($\text{ng N soil g}^{-1} \text{ h}^{-1}$)
Main effect	Intercept	17.7 \pm 1.0 ***	88.8 \pm 3.8 ***	153.8 \pm 76.1 *
	Residue	18.1 \pm 1.4 ***	44.1 \pm 5.4 ***	833.1 \pm 107.6 ***
	Earthworm and Residue	16.9 \pm 1.4 ***	45.6 \pm 5.4 ***	1060.5 \pm 107.6 ***
	Cu	-4.5 \pm 1.2 ***	-61.4 \pm 4.2 ***	-134.1 \pm 76.5 *
	Heat	-6.1 \pm 1.2 ***	-49.2 \pm 4.2 ***	-136.1 \pm 76.5 *
	Day	0.0 \pm 0.1	-0.7 \pm 0.2 **	9.5 \pm 3.8
Interaction effect	Residue \times Cu	-7.3 \pm 1.6 ***	-3.0 \pm 6.0	-753.8 \pm 108.3 ***
	Earthworm and Residue \times Cu	-6.9 \pm 1.6 ***	-11.1 \pm 6.0	-1006.2 \pm 108.3 ***
	Residue \times Heat	-10.7 \pm 1.6 ***	0.8 \pm 6.0	-701.3 \pm 108.3 ***
	Earthworm and Residue \times Heat	-8.6 \pm 1.6 ***	-6.2 \pm 6.0	-929.6 \pm 108.3 ***
	Residue \times Day	-0.4 \pm 0.1 ***	0.5 \pm 0.3	32.9 \pm 5.3 ***
	Earthworm and Residue \times Day	-0.4 \pm 0.1 ***	0.2 \pm 0.3	11.1 \pm 5.3 *
	Cu \times Day	0.0 \pm 0.1	0.5 \pm 0.3	-10.0 \pm 5.3
	Heat \times Day	0.1 \pm 0.1	1.0 \pm 0.3 ***	-3.5 \pm 5.3
	Residue \times Cu \times Day	0.1 \pm 0.1	-0.7 \pm 0.4	-30.6 \pm 7.5 ***
	Residue and Earthworm \times Cu \times Day	0.2 \pm 0.1	-0.7 \pm 0.4	-6.8 \pm 7.5
	Residue \times Heat \times Day	0.3 \pm 0.1 **	0.0 \pm 0.4	6.0 \pm 7.5
	Residue and Earthworm \times Heat \times Day	0.3 \pm 0.1 **	0.4 \pm 0.4	15.6 \pm 7.5 *
	Fixed effect R^2	0.82	0.90	0.87
	Random effect R^2	0.01	0.01	0.02

*Note: the structure of linear mixed model: function ~ Field treatment * Stress * Day + Random effect from replicates.*